A NEW METHOD OF DETERMINING DIETS OF RODENTS

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Diets of rodents often have been studied by analysis of feces or stomach contents. Analysis of feces is highly inaccurate because of differential digestion, and the analysis of stomach contents requires the sacrifice of large numbers of individuals and cannot be used for some species because of conservation considerations. We developed a new method for determining diets of rodents by stomach pumping that is as accurate as previously used analysis of stomach contents. Animals are not sacrificed, so it is possible to collect more than one sample from each individual and study a population of rodents for a long time without harming it. It also allows studies that would otherwise be legally or morally impossible.

Key words: *Acomys,* spiny mice, diet, feces, stomach contents, stomach pumping, food habits, Israel

Diets are extremely significant for determining evolution, life-history strategies, and ecological roles of animals. Food is one of the most important dimensions of the niche, and therefore, information on diets of animals is virtually a prerequisite for most ecological research (Krebs, 1989). Study of diets of animals is crucial for understanding relationships between species (Bar et al., 1984; Zimmerman, 1965) and between an animal and its environment. These relationships may determine community structure, species diversity, relative abundances, and resource partitioning among species and individuals (Connell, 1975).

The study of food habits also is important for conservation, such as planning nature reserves, understanding consequences of introducing game animals (Cole et al., 1995), reintroducing locally extirpated species to their natural habitats (Bright and Morris, 1994; Hartman, 1994; Lundie-Jenkins et al., 1993; Renssen and Vogel, 1993), and developing biological control (Beg et al., 1994; Jimenez et al., 1994; Tobin et al., 1994).

Diets of rodents usually are evaluated by analyzing stomach contents or, less frequently, fecal material. Both methods have disadvantages. The main disadvantage of fecal analysis is that food items have passed the entire digestive system. Different food items are differentially digestible, which may bias results of fecal analysis (Bergman and Krebs, 1993; Dickman and Huang, 1988; Hansson, 1970; Jeuniaux, 1961; Neal et al., 1973). Analyzing stomach contents demands sacrificing animals, often in large numbers. For example, previous studies have involved killing 315 individuals of gerbilline rodents (Bar et al., 1984), 275 individuals of several small species of mammals (Kerley, 1992), 2,881 individuals of several species of rodents (Reichman, 1975), 186 collared lemmings (*Dicrostonyx kilangmiutak*) and tundra voles (*Microtus oeconomus*—Bergman and Krebs, 1993), 1,675 roof rats (*Rattus rattus*—Tobin et al., 1994), and 1,222 house rats (*Rattus rattus*—Mushtaq et al., 1995). Therefore, this method cannot be used for the study of species with small populations that are under threat of extinction or protected by law. Currently, an increasing number of species are in these categories.

We describe a new method of determining diets of rodents by stomach pumping; animals are not sacrificed, so it is possible to collect more than one sample from each individual, and it is possible to study a population of rodents for a long time without harming it. We compare results obtained by

this method with those obtained from analysis of stomach contents and feces.

Materials and Methods

Six adult common spiny mice (*Acomys cahirinus*) from a laboratory colony at the Meir Segals Zoological Garden at Tel Aviv University (mean body mass 42 ± 4 g) were kept in individual cages (35 by 45 by 22 cm), outdoors (April), under natural photoperiod and temperature. We offered them a mixture of food types representing their assumed natural diet (Degen et al., 1986): barley (representing seeds), lettuce (as green matter), snails (*Sphincterochila prophetarum*—the species that is eaten by the mice in the field) collected at Ein Gedi, and crickets (representing arthropods). Food types chosen were those preferred in each category in a preliminary experiment in which mice were offered different types of seeds, lettuce, green alfalfa, and a few species of arthropods. All food types were offered ad lib. and replaced every 24 h. The amount of food required per animal to feed ad lib. over a 24-h period was determined through a preliminary experiment.

After 4 days on this diet, we collected fresh feces from each individual and performed stomach pumping. Individuals were anaesthetized by placing them in a jar with a pad of commerical pharmaceutical cotton wool soaked with ether. We inserted a flexible plastic tube through the esophagus of the anaesthetized mice. The tube originally was produced as a catheter for an intravenous placement unit (external diameter = 2 mm) with the leading edge cut at an angle. We injected 1–2 ml of saline through the tube with a syringe. We took a sample by drawing a similar amount of liquid from the stomach. Mice returned to normal activity within a few minutes. We kept the same individuals on the same diet under the same conditions for another week and then killed them (using CO_2 pumped into a glass jar) and collected their stomach contents. Thus, the three methods of assessing diets were studied on the same six individuals, kept under the same conditions, within a week.

We prepared stomach contents, stomach-pumped material, and feces by a technique similar to Sparks and Malechek (1968) and Hansson (1970). Samples were mixed thoroughly, and a few drops were placed in a nine-well glass plate. The samples were washed with distilled water and placed in a well containing a few drops of hematoxylin (Hansson, 1970). After 1 min, samples were washed again and placed on a microscope slide. Hoyer's solution (Baker and Wharton, 1952) was added, and a cover slip was placed on the sample. The slide was dried at 30°C for several days. Three slides were prepared from each sample. The number of slides was chosen after a preliminary experiment (Bar, 1983), in which we calculated mean abundance of the most common dietary item using two slides, then three, and then four. We chose to use three slides because we found that adding a fourth slide altered the mean <4%.

TABLE 1.—*Relative abundance (%) of four foods in the diet of the common spiny mouse (*Acomys cahirinus*) as estimated with three methods (n = 6, results presented as $\bar{x}$ ± SE).*

Food type	Method		
	Feces	Stomach contents	Stomach pumping
Snails[a]	9.4 ± 5.8	23.7 ± 10.3	24.0 ± 7.8
Crickets	0 ± 0	19.1 ± 5.8	19.3 ± 7.8
Lettuce	41.7 ± 17.9	26.0 ± 12.3	24.6 ± 11.7
Barley	48.8 ± 14.6	30.1 ± 5.5	32.0 ± 14.7

[a] *Sphincterochilia prophetarum.*

We estimated relative abundances of the four food types using a microscope at 30× magnification. The microscope ocular had a grid containing 100 quadrates. On each slide, we counted the number of quadrates that contained fragments of the four food categories for the entire slide. We calculated relative abundance of a given type of food by dividing the number of quadrates that contained fragments of that food category by the total number of quadrates containing any food type. To test differences between food types and methods, we performed a one-way analysis of variance (ANOVA) with repeated measures (food types and methods) on arcsin-transformed data (Zar, 1984). To test which method differed, we performed a one-way ANOVA with repeated measures on only two of the methods.

Results

We found insect remains in stomach contents and in stomach-pumping material, but none in feces (Table 1). Therefore, we performed the one-way ANOVA using only the three other types of food. The one-way

ANOVA with repeated measures performed on results of the three methods revealed a significant ($P < 0.05$) difference between types of food, a significant difference ($P < 0.05$) between methods, and no significant interaction ($P > 0.05$). A one-way ANOVA with repeated measures on two of the methods (excluding feces analysis) revealed no significant difference ($P > 0.05$) between types of food, no significant difference ($P > 0.05$) between methods, and no significant interaction ($P > 0.05$). Thus, fecal analysis was significantly different from the two other methods.

Discussion

Studies of rodent diets commonly have assessed the importance of different food items in terms of their relative abundance in stomach contents or feces (Begg and Dunlop, 1985; Bergman and Krebs, 1993; Dickman and Huang, 1988; Kerley, 1992; Kerley et al., 1990). We wished to overcome disadvantages of both methods and develop an alternative. Our stomach-pumping method gives the same results as stomach-content analysis; it amounts to removing the stomach contents orally and, therefore, does not harm research animals and permits long-term study of individuals and populations.

Results of fecal analysis differed significantly from the results of the two other methods, and this may be due to different digestibility of food items. It has been demonstrated previously that some ingested items (like small or soft-bodied insects or easily digested plant materials) digest completely, leaving no evidence in the feces, or are so digested that they become unidentifiable because their diagnostic characters are obscured and hence are underestimated in the diet (Bergman and Krebs, 1993; Dickman and Huang, 1988; Hansson, 1970; Jeuniaux, 1961; Neal et al., 1973). Underestimation of insects in feces may occur in species that produce chitinases in the gastric mucosa (Jeuniaux, 1961). Dickman and Huang (1988) found such underestimation in insectivorous small mammals. If soft-bodied insects are eaten, they may be recognizable in stomach contents but not in feces (Kunz and Whitaker, 1983). Neal et al. (1973) compared fecal and stomach-content analyses in the meadow vole (*Microtus pennsylvanicus*) and suggested that results of food-habit studies based solely on analysis of fecal material should be treated with caution, because species of plants with fragile cuticles may be considerably underestimated and species of plants with cuticles resistant to digestion may be overestimated.

We found no remains of insects in feces. Jeuniaux (1961), checked the existence of chitinase in eight species of vertebrates and found it in six, all at least partly insectivorous. *A. cahirinus* is an omnivore whose diet includes chitin-covered prey, as do those of many species in all major rodent lineages (Landry, 1970). It is a tenable hypothesis that these species digest chitin; it would be interesting to study this possibility.

Recently, we have been carrying out field research on food habits of two species of spiny mice (*A. cahirinus* and the golden spiny mouse, *A. russatus*) using this method, and we have found it easy to employ in the field without removing animals from the point of capture. We trap mice in Sherman live traps but make the bait unavailable for consumption (by placing it in a small wire bag), or use only drops of vanilla extract in traps to attract the rodents. The entire stomach-pumping procedure takes no more than a few minutes, and animals recover quickly and suffer no evident ill effects. We have been able to recapture the same individuals and sample them at different seasons without harming the population. This has enabled us to conduct our study of food habits as part of a long-term ecological study.

Using this method, we were able to analyze ratios of different food types in diets of field-trapped individuals and draw conclusions regarding the dietary basis of our study species. Direct observations revealed that spiny mice usually removed taxonom-

ically useful heads, legs, and wings of their arthropod prey before consumption and, thus, often render identification to family or genus impossible, but the same difficulty would have been encountered if we used stomach contents.

This method has enabled us to study food habits of desert rodents (*A. cahirinus* and *A. russatus*) that are protected by law. Their population densities are low and their populations at Ein Gedi are smaller than those that have been sacrificed in many stomach-contents studies. We are able to study our research populations without sacrificing them. Stomach pumping should be equally applicable to the study of populations of other species of rodents and can allow studies that would otherwise be legally or morally impossible.

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